II

GONOCOCCUS ECTO-ANTIGEN *

By JOHN O. OLIVER, M.B., B.S.(Lond.), M.R.C.S.(Eng.), L.R.C.P. (Lond.); Lecturer in Pathology and Bacteriology and Assistant Pathologist, St. Thomas's Hospital.

DR. CLEMENTS has stressed what we believe to be the two most important factors in the treatment of gonorrhœa, namely, free drainage and a sufficiency of blood reaction as indicated by the complement fixation test for the gonococcus.

Dr. Clements has mentioned the use of a special type of vaccine, which we call " ecto-antigen," as the means whereby the necessary degree of blood reaction has been attained.

This " ecto-antigen " is no new product, and is most certainly no discovery of mine. The name " ecto-antigen " is one which we have applied to that special vaccine which is the result of the researches of Major Lyn Dimond, late of the R.A.M.C., and called by him gonococcus " exotoxin." The first paper on this subject appeared in the *Journal* of the R.A.M.C. for March, 1927, when Majors Lambkin and Dimond, with Captain Robinson, communicated a preliminary report on " the employment of certain constituents of the gonococcus in the treatment of gonorrhœa and other constituents in tests of 'cure.'" Subsequent to this a paper was read by Major Lambkin and Major Dimond at the British Medical Association Meeting in July, 1927, and most of you will remember that Major Lambkin read a paper to this Society in November, 1926, on the same subject. The work reported in these papers was carried out at the Royal Herbert Hospital, Woolwich, and I was privileged in the very early days of the institution of this treatment to be allowed to see the methods used at Woolwich, both in the preparation of the vaccine and its use. It was due entirely to the kindness and help which I received from Major Lambkin and Major Dimond that I was able to work at this subject at all.

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I have, therefore, worked with this peculiar preparation since 1926. For some time the work on the clinical side was necessarily somewhat spasmodic, but some two years ago, due to the interest and kindness of Colonel Harrison, the assistance of the Ministry of Health was obtained, and the provision of a grant enabled the work to be carried out systematically.

Although this evening our essential claim is a method of treating gonorrhœa based on a belief in the necessity for free drainage, and the obtaining of considerable blood reaction, yet this is coupled with the use of gonococcus " ecto-antigen," because up to the moment we have found no other means as reliable and efficient in producing the necessary blood changes. I ask you to forgive this somewhat long preamble, because I have wished to make clear one point which I think may arise in the minds of some of you. That is, why, if the gonococcus vaccine is so efficient and so reliable, and also, if it has been described and reported on for so long a time, has its use not become more general ? My personal experiences will explain, I believe, this point and provide the answer. Although I have been working with this vaccine for seven years, yet even now I find it to be one of the most tricky and difficult preparations that a bacteriologist is likely to be called upon to contend with. Even now, I cannot claim to know a great deal more about the preparation than when I began. In many ways the peculiar behaviour of the gonococci grown in the manner necessary for the production of this gonococcus " ecto-antigen " is as big a mystery as ever, and as time goes on I have more and more regard for the brilliant original research work of Major Dimond.

The difficulties, disappointments and actual failures experienced in the making of this vaccine are likely to prove almost complete stumbling-blocks to the majority of those who might attempt the manufacture, and would, I am sure, have proved equally so for me, had it not been for the help which I received originally, and which I have already acknowledged.

I do not propose to weary you with any long description of the gonococcus " ecto-antigen." Essentially the gonococcus " ecto-antigen " as I make it to-day is the same preparation as the gonococcus exotoxin originally prepared by Major Dimond. The mode of employment

has been considerably modified. The other products, such as the gonococcus "endotoxin," which originally played a part in the treatment advocated by Lambkin and Dimond, have disappeared from our work altogether. Indeed, as those of you who remember the original papers will agree, we have attempted to simplify, to the greatest possible degree, the adjuvant treatment necessary when using "ecto-antigen" in order to try, as far as possible, to gain clear evidence of the results of the use of thsi special form of vaccine.

Very briefly and merely for the sake of completeness, I will outline the method of preparing the vaccine.

Special strains of gonococci are necessary, and these are such as will grow very freely on ordinary laboratory media. This means many sub-cultures of the average strain of recently isolated gonococci. As you are all aware, gonococci require a rich culture medium for their growth when freshly isolated, but after numerous sub-cultures they can be so changed that in many cases they will grow on ordinary agar without the addition of any enriching substances. Such strains as these are absolutely necessary for the present work.

The actual "ecto-antigen" consists of a solution of certain protein metabolic products of the gonococcus in 2 per cent. carbol-saline (Fig. 1). These products are

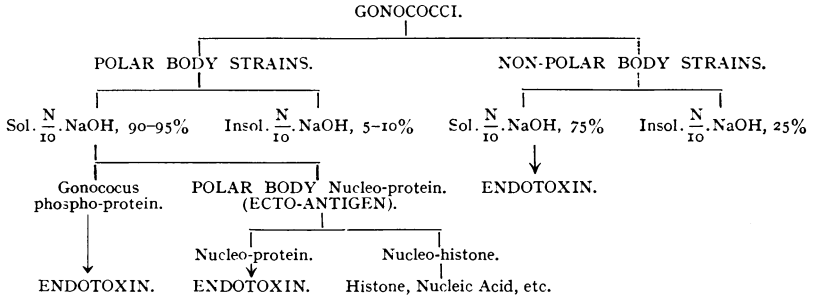

FIG. 1.

obtained by growing the gonococcus in such a way that the organisms extrude a "polar body." This polar body, stainable with the Neisser's method of staining as for the polar bodies of the diphtheria bacillus (Fig. 2), was originally described by Babe and is often known by his name. To obtain the production of these polar bodies, a special medium is necessary for the growing of the

gonococcus. This medium is a solid one, with an agar base, is enriched with fresh beef-heart extract as well as with fresh unheated human serum, and the whole is made especially rich in nuclein and nuclear substances, containing nucleic acid, desiccated thymus gland, and extract of ripe herring roe. It is necessary to prepare this medium in such a way that, when finished, its pH shall be exactly 7·2, and no artificial alteration of reaction of the mixed ingredients is permissible. This medium is a difficult one to prepare, and the greatest possible care and pre-

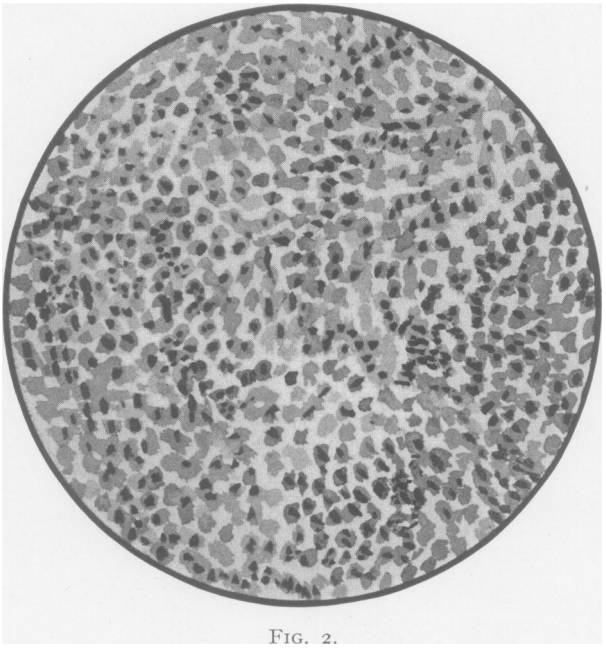

FIG. 2.

cision is necessary if various batches are to give comparable results.

Apart from the stimulation of the gonococcus to produce "polar bodies," this medium has a further function. It is so made that actual detoxication of the gonococcus growth occurs during culture, and toxic substances are precipitated into the surface of the medium.

This medium is inhibitory to the gonococcus when freshly isolated, and it is for this reason that strains of gonococci which have become accustomed to a poor medium are necessary; not that the present medium is

poor in nutrient material, but that it is definitely inhibitory to the growth of newly-isolated strains.

Growth is allowed to take place on this medium for exactly twenty hours, neither more nor less being permissible. During this period of incubation the medium will become very slightly more alkaline on its surface, as indicated by the very slightest possible change of colour of the medium, in which phenol-red is incorporated for this purpose. Prolonged culture leads to the whole of the medium becoming alkaline, due to the metabolic products of the gonococcus. It is essential for proper antigen production that the medium should remain at an almost constant pH during the growth of the organisms. A suitable strain of gonococci grown on this medium will show at the end of twenty hours' incubation a profuse growth of a dry character. A small amount of this culture will emulsify quite readily in saline or water, and can be spread on a glass slide almost like a fragment of the culture of a staphylococcus. This is in marked distinction to what is found in the average gonococcus culture grown at a rather higher pH, when the growth is definitely of a mucoid type.

The culture, when obtained in this way, is washed off with 2 per cent. saline, to which 0·5 per cent. carbolic acid has been added. The suspension is then centrifuged at high speed, and a really good centrifuge is necessary for this purpose, giving a minimum of 10,000 revolutions per minute. The clear supernatant fluid after this process is of a slightly buff colour, and forms the gonococcus " ecto-antigen."

Time will not permit me to deal more fully with the laboratory technique for the production of gonococcus " ecto-antigen." The collection of good strains is a most important point, and I believe that I have solved this problem to a considerable extent by the study of gonococcus cultures grown over a period of twenty to twenty-eight days on a suitable thick culture medium, the colonies being studied photographically; in this way it is possible to pick out strains which will give almost 100 per cent. of " polar body " production quite quickly, instead of the necessity for innumerable sub-cultures over a period of months or years which will otherwise be found the only way of obtaining cultures which will give a reasonable yield of the gonococcus " polar body."

The serum used for enriching the culture medium is obtained, for convenience' sake, from patients attending the V.D. centre. It was throughout known to be inadvisable to select a case which had previously suffered from gonorrhœa, and that syphilitic, non-venereal cases, or very early acute gonorrhœas, were the more suitable. I have been able to trace the reason for this preference to the fact that if a serum is used which has been obtained from a patient who has any considerable blood response to the gonococcus, as indicated by the complement fixation test, this serum will prove definitely inhibitory to the production of " polar bodies." For this reason it is now my practice to test all serum which is to be used in the manufacture of batches of medium by the gonococcus complement fixation test. Serum is used only if it gives a cleanly negative result in this reaction.

Before concluding, I should like to say a few words regarding experiments made with the gonococcus " ecto-antigen," as I believe that these may provide some ground for the discussion which is to follow these papers.

Firstly, in regard to the actual vaccine and the use of it by the intra-dermal route. The " ecto-antigen " is, as I have said, made up as a solution in 2 per cent. saline to which 0·5 per cent. carbolic acid has been added. The result is a solution which, if it gains access to a blood-vessel, will produce immediate thrombosis. This is undoubtedly a danger if the vaccine is to be used for any form of deep injection, and was originally one of the reasons for the choice of the intra-cutaneous route. The choice was, however, a most happy one, because I believe that there is little doubt that, apart from a general reaction—and that this occurs is well indicated by the blood response—a definite cutaneous immunity as visualised by Besredka is established. If this is so, then one can well understand beneficial results in the cutaneous and semi-cutaneous structures with which we deal in gonorrhœa.

I cannot take up your time by illustrating the results of my animal experiments, and I would say only that I conclude from them that the action of gonococcus " ecto-antigen " administered by the intra-cutaneous route is definitely one upon the reticulo-endothelial system of the body.

One of the most interesting points observable from the

bacteriological point of view in the treatment of cases of gonorrhœa by means of gonococcus " ecto-antigen " is the very suddenness with which gonococci are found to disappear ultimately from the discharges. In the examination of some thousands of smears, I have been struck over and over again by this point ; and not only by this point, because there is another and one which is, I believe, of the utmost importance, namely, that whereas under the usual conditions of treatment gonococci gradually become more and more difficult to find in smear preparations, in this method gonococci remain quite easily found until the moment of their disappearance, and then disappearance tends to be complete and absolute and to occur, as I have already said, quite suddenly.

The ease with which gonococci can be found in a smear from a case under treatment with " ecto-antigen," if the organisms are present at all, is truly remarkable, and for this reason experience has shown me that if gonococci are not found after a short search in such cases, they are unlikely to be found at all. This is, and I am sure you will agree with me, in marked contrast to what is found under other conditions of treatment.

One other point of interest from the aspect of " cure " is in regard to the fall in the blood response as indicated by the complement deviation test. In a paper written some years ago, by Osmond and myself, we showed that in cases under routine treatment for gonorrhœa positive complement deviation tests appear to fade away extremely slowly, and we concluded that, from the point of view of " tests of cure," a falling reaction could be assumed as satisfactory. As a result of my work with gonococcus " ecto-antigen," I am more doubtful as to whether this is really the case. With apparent " cure " in a case treated with gonococcus " ecto-antigen," the blood reaction is found to fall away very rapidly indeed, and I rather wonder, therefore, whether the slowly falling reaction so often seen in cases of gonorrhœa treated by other means may not in some cases represent a gradually lessening response to the constant stimulus of a few remaining organisms, deeply embedded in the tissues.